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越南地区柑橘黄龙病发生及防治

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摘要：黄龙病是全球广泛发生的毁灭性病害，柑橘木虱为其主要媒介昆虫。越南最早于上个世纪六十年代有相关报道。通过一些国际合作项目，越南对柑橘木虱和黄龙病进行了一系列研究并积极采取措施有效防控：包括消除病株、筛选抗病虫品种、改变种植密度和时间、化学防治与生物防治、作物间种、喷施矿物油乳剂、施用有机肥、使用无病苗和无病接穗并在运输过程中封闭保护等。其中控制黑臭蚁 *Dolichoderus thoracicus* **增殖黄猄蚁** *Oecophylla smaragdina*、**交错式间种番石榴以控制柑橘木虱和黄龙病值得中国借鉴和参考。**

关键词：黄龙病；柑橘木虱；番石榴间种；黄猄蚁；矿物油乳剂
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Huanglongbing occurrence and management in Vietnam

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Abstract： Huanglongbing（HLB）is a destructive citrus disease in almost the world. In Vietnam，the disease has been reported since early 1960s，and it is caused by Gram negative bacteria "*Candidatus* Liberibacter asiaticus"，which is transmitted by the Asian citrus psyllid（ACP），*Diaphorina citri*. Through many international projects，Vietnam has studied on it and the control measures against it，including removing diseased trees，selecting resistant varieties，changing time and density of citrus planting，intercropping，chemical and biological control，spraying mineral oils，supplying organic fertilizer，utilization of pest free seedlings and grafting scions and avoiding freely moving the citrus seedlings in open air，etc. Controlling the black ant *Dolichoderus thoracicus* for better cultivation of the weaver ants，*Oecophylla smaragdina*，and interplanting of citrus with guava as staggered pattern might be used for reference to control HLB and ACP in China.

Key words： Huanglongbing；*Diaphorina citri*；intercropping with guava；weaver ant；mineral oil

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1 Introduction

In Vietnam, citrus is an important fruit crop and gives a very high yield for grower. There are about 220 000 ha citrus planting in 2017 (Source from Ministry of Agriculture and Rural Development, 2017). However, many citrus pests and diseases cause severe damages in Vietnam, and Huanglongbing (HLB) is the most dangerous and destructive diseases.

The control measures against HLB have been studied under many international projects in Vietnam since early 1960s. The results are summarized and revealed in this paper.

2 HLB occurrence in Vietnam

HLB is caused by gram-negative bacteria "*Candidatus* Liberibacter" (Jagoueix *et al.*, 1994; Bové, 2006). At least three non-cultured species of "*Ca.* L." genus have been implicated in HLB in Asia, Africa and the Americas (do Carmo Teixeira *et al.*, 2005; Gottwald *et al.*, 2010). "*Ca.* L. asiaticus" tolerates high temperatures and can live at temperatures above 30℃ for long periods of time, but "*Ca.* L. africanus" is restricted by high temperatures and manifests at temperatures between 16 ~ 22℃, and "*Ca.* L. americanus" is sensitivity to temperature which is similar to "*Ca.* L. africanus" (Garnier *et al.*, 2000; Bové, 2014). South China was regarded as the first source of this disease in Asia (Reinking, 1919), but previous descriptions suggested that the disease was present first in India (Capoor *et al.*, 1967; European Food Safety Authority (EFSA) *et al.*, 2019). Beattie *et al.* (2006) hypothesized that the disease was originated in Africa, possibly from an asymptomatic host such as *Verpris lanceolata* .

The bacteria can be transmitted by citrus psyllid and they can multiply in the insect. The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama is considered a vector of HLB in Asia (Capoor *et al.*, 1967; Martinez and Wallace, 1967; Lou, 2018) and *Triozaery treae* Del Guercio in Africa (McClean and Oberholzer, 1965; Yamamoto *et al.*, 2006).

In Vietnam, HLB is also called as golden leaf disease, which is caused by "*Ca.* L. asiaticus", and *D. citri* is its vector. HLB was first reported in the early 1960s (Ha, 1991). HLB spreads very quickly, destroys many orchards and causes serious damage to citrus production in Vietnam. Many farmers have removed and replanted with other crops, while other growers have continued to replant citrus but have not followed the basic principles such as removing diseased trees, controlling ACP and using pest free seedlings and grafting scions to reduce the risk of re-infection and damage.

The disease had been occurring since the 1960s with a prevalence of 1% to 5%, mainly due to citrus seedlings propagated through extraction from diseased plants (Ha, 1991). After 1975, in the Northern Provinces, the disease increased up to 60% ~ 100%. Of which, 30% ~ 40% was completely out of productivity. In one orchard, more than 300 trees were infected with HLB from a few infected seedlings in 84 months.

In 1996, a survey on HLB infected degree in "yellow-orange" orange orchards with different age was conducted in Tam Binh, Vinh Long. The result showed that younger citrus trees were easier to be infected by HLB bacteria (Table 1).

Table 1 HLB infected percent in orange orchards with different age

Orchard age (y)	HLB infected percent (%)	Area of surveyed orchard (ha)
4 ~ 5	80.00	409
5 ~ 8	20.00 ~ 50.00	276
8	1.00 ~ 5.00	49

Note: * Source from Ministry of Agriculture and Rural Development, 2017.

At the end of 2004, Phan Thanh Tri and his co-workers carried out a survey in several citrus-growing provinces such as Tien Giang, Vinh Long, Can Tho and Dong Thap. The results were showed as Table 2.

Table 2 Level of HLB infection in citrus-growing provinces

District	Citrus species	Level of HLB infection	Orchards without HLB (%)
Tien Giang: Cai Mau, ChauThanh	pomelo, orange	+ + + + + 32.4%	0
Vinh Long: Binh Minh	pomelo	+ + +32.7% , + +27.4%	31.5
Vinh Long: Tra Vinh	orange	+ + + + + 28.5% , + + + +53%	0
Vinh Long: Tam Binh	orange	+ +55%	0
Can Tho: Long Tuyen	lemon	+ +58.06% , +19.4%	6.5
Dong Thap	tangerine	+ +9.4%, +15.6%	75.00

Note: * Source from Ministry of Agriculture and Rural Development, 2017

3 Huanglongbing management in Vietnam

The Southern Horticultural Research Institute (SOHRI) has been cooperating with many international organizations in researching of pest management and restoring the citrus industry. The first survey conducted by Dr. Aubert and Bourdeaut from the French Agricultural Research Centre for International Development (CIRAD) in the citrus growing areas in Can Tho and Tien Giang and the disease was officially announced at the first conference held at Long Dinh Fruit Research Center, the predecessor of Southern Fruit Research Institute (SOFRI) and SOHRI, followed by a survey by Prof. Bove and Dr. Garnier from the French National Institute for Agriculture Research (INRA). In this episode, the scientists reiterated that the disease was caused by Gram negative bacteria "*Candidatus* Liberibacter" and transmitted by *D. citri* from diseased plants to healthy plants. Then, with the help of CIRAD, the Southern California Institute of Crop Research has produced a series of disease free headwaters that provide locality for the top seedlings to serve local communities in the area. At present, the institute cooperates with international organizations, including CIRAD, Australian Centre for International Agricultural Research (ACIAR), Food and Fertilizer Technology Center (FFTC) and Japan International Research Center for Agricultural (JIRCAS) to try to find out the most effective management measures.

3.1 Removing diseased plants

Eliminating dangerous pathogens by removing diseased plants in citrus orchards is a very effective treatment to control HLB. But as analyzed above, pathogens are not only present in the symptomatic trees but are more present in the plant that do not show signs or symptoms. These trees should be identified early and accurately. It is a major issue that needs to be addressed. And even more, the diseased trees are very difficult to be removed by many citrus growers if they continue to produce fruit.

3.2 Disease and psyllid free seedlings and graft scions

Disease free seedlings have been used in many citrus growers, particularly successful in China (Lin and Ke, 1986). In Vietnam, especially in the southern provinces, the production of disease free seedlings through the top grafted seedlings combined with storing in a two-doors net has been implemented since 1996 at the Southern Fruit Research Institute, supplying mother trees for seed centers in Tien Giang, Ben Tre, Vinh Long and other districts for the purpose of propagation of citrus seedlings of 26 varieties. In Vietnam, the ordinance on plant varieties had been issued. It was suggested that freely moving the citrus seedlings in open air should be avoided. The cutting tools should be cleaned by Javel/Sodium hypochloride solution when move from one plant to others to avoid mechanical transmission. Treatment of citrus seedlings with pesticides of Imidacloprid, Thiamethoxam or Clothianidin, prior to planting about 10 days, is very good seedling protection and effective ACP management (Gottwald *et al.*, 2010).

However, the application in the production and management of citrus seedlings has not been fully implemented. Therefore, the production of citrus orchard floating is still rampant, not manageable.

3.3 Biological control of citrus psyllid

Psyllid nymphs may be parasitized by the parasite wasps *Tamarixia dryi*, *T. radiate* and *Diaphorencyrtus aligarhensis*. Good orchard management is suggested to preserve the high population of these natural enemies in Vietnam.

The tree-inhabiting weaver ants, *Oecophylla smaragdina*, protect tropical tree crops effectively as they patrol canopies actively and prey upon or deter a wide range of potential pests. Vietnamese citrus farmers cultivate population of weaver ant for citrus pest control. Up to 20% of them do not use any pesticides and there are few citrus psyllids and HLB diseased trees in their orchards (Fig. 1).

Fig. 1 The application of *Oecophylla smaragdina* in the citrus orchards in Vietnam

Although weaver ant husbandry is a centuries-old tradition in China and Vietnam, scientific research on it started in China after problems of insecticide resistance (Yang, 1982; Huang & Yang, 1987; Yang, 2002) and in Vietnam since the 1990s. Weaver ants were reported to control a range of pests, including the citrus stinkbug *Rhynchocoris humeralis*, the aphids *Toxoptera*, leaf-feeding caterpillars *Papilio*, inflorescence eaters, coleoptera and various other pests. By contrast to the above examples, research on *Oecophylla* in citrus was driven by national scientists working closely with farmers, their culture and their knowledge system. Due to pressure from the pesticide industry, the use of weaver ants in citrus orchards decreased during the 1990s. However, various small-scale financial injections made from the mid 1990s onwards, media coverage and involvement of farmer associations have reversed this trend (N. T. T. Cuc, personal communication). Although competition between dominant ant species proved a key challenge to researchers in Africa, experienced Vietnamese citrus farmers developed ingenious ways to optimize the performance of weaver ants. They trap the black ant *Dolichoderus thoracicus* and avoid the intercropping of sapodilla fruit trees, because black ants favor these trees as nesting habitat. Newcomer of citrus planting who naively introduced sapodilla as an intercrop to diversify their source of income because this fruit tree requires little care. Under competition from the black ant, natural pest control by *Oecophylla* in citrus

orchards was disturbed.

Paecilomyces fungus has been investigated in laboratory conditions which show very high prevention efficiency. It is suggested that Paecilomyces should be applied in citrus orchards at early stage (at first 7 months after planting).

3.4 Intercropping with other crops

Besides wind break plants, guava-citrus interplanting is more generally adopted in citrus orchards in Vietnam.

Guava, *Psidium guajava* Linn is a widely planted tropical and subtropical fruit. Guava leaf is traditionally used as an antidiarrheal drug. This suggests that use of guava chemicals as insecticides will be safe to mammals. It was reported in Vietnam that HLB infection reduced when citrus intercropping with guava (Fig. 2). Since a long time ago, farmers live in Mekong Delta of South Vietnam have been practicing interplanting of citrus with guava and there was much lower psyllid density and HLB incidence compared to citrus orchards lacking guava (Hall *et al.*, 2007). Guava trees were generally planted antecedent to citrus planting and the two plants were interplanted at a ratio of one guava tree to one citrus tree as staggered pattern in Vietnam (Beattie *et al.*, 2006).

This practice adopted by Vietnamese farmers drew the attention of scientific communities of many countries during 1990s and various scientific studies were initiated to understand the scientific reasons. The first of this kind of research was a collaborative research projects involving Vietnamese, Japanese and Australian scientists (Beattie *et al.*, 2006). The findings of this research which was presented in a meeting held during December 2006 in Japanese International Research Center showed that interplanting citrus with guava negated infestations of Asian citrus psyllid and consequently HLB (Beattie *et al.*, 2006). This study collected the scientific information that young citrus interplanted with guava remained disease-free for a year whereas sole citrus crop showed signs of the disease within four months of planting and reached over 30% trees infected within a year (Hall *et al.*, 2007). A team of American scientists who also made an observation tour to Vietnam in April 2000 noted that the normal life of sole citrus plantings in Mekong region of Vietnam was 2 to 4 years, but those interplanted with white guava were surviving for up to 15 years (Gottwald *et al.*, 2010). Gottwald *et al.* (2010) argued that guava can effectively banish psyllid 1 to 1.5 years after planting, and in Thailand, the use of intercropping with guava is also good. Other studies in Florida and Brazil are not effective because more than half of guava cannot live in cold conditions in these areas.

Fig. 2　The citrus orchard intercropping with guava in Vietnam

It was postulated that the effects of guava on citrus psyllid could be due to mechanical/physical disruption on host recognition, repellent effect of volatile compounds from guava or chemical alteration of the volatile compounds emitted by citrus reacting with guava compounds. Gottwald *et al.* (2010) noted high adult mortality rates occurred when psyllid were confined to guava in no-choice situations, with 95% mortality occurring within 6 ~ 9 days. They postulated that the effect may be due to volatile compounds

produced by guava are deleterious to psyllid.

Neuman *et al*. (2011) studied the use of volatile compounds from guava for biological control against psyllid. In theory they are highly effective, but it is difficult to apply in practice because the evaporation effect of synthetic compounds spraying must be applied continuously and the cost is very high on large scale. More research is needed to identify the solution. Therefore, the intercropping in the orchard is the better solution because of continuous, long-term effect and most economical.

Zaka *et al*. (2010) evaluated the repellent effect of guava leaf and factors attributed to this activity, response of adult psyllid to guava leaves and its odor in cage test and Y-tube alfactometer test. The alfactometer response of both male and female psyllid responded similarly to the guava leaf odor and the repellent effect of guava volatile against citrus psyllid was dose-dependent. Low doses had little effect on citrus psyllid. This result indicates that sufficient number of guava trees is needed to keep the dosage of volatile compounds emitted from guava at an effective level in the entire orchard. Besides, guava interplanting may increase severity of fruit fly damage to citrus (Xie & Zhang, 2005; Lin *et al*., 2005).

3.5 Proper nutrition prolongs the life of citrus

Razi *et al*. (2011) defined the role of nutrition in reducing HLB. Spraying of micronutrients is considered the current solution that increases resistance of plants and fruit yield. Xia and Sequeira (2011) reported the application of nutrient solutions in HLB management. Utilizing a variety of micronutrients as part of disease management solution prolonged the life of the diseased plant in Florida. This solution had been applied in China long before. The results of more than 60 years of experiments in the field showed that nutrients contributed greatly to increasing resistance to HLB and prolonged harvesting time from diseased plants (Park *et al*., 2019).

Increasing organic and inorganic fertilizers had better effect in orange trees in Vietnam. It is suggested in Vietnam that supply more organic fertilizer for better healthy plant especially useful microorganism like *Trichoderma*/*Streptomyces*. Nutrition is mixed with pesticide and sprayed 3 times on the main leaves of young trees for high effectiveness. Under optimum nutrition conditions combined with rational irrigation, a 10-year-old tangerine tree will last for 3 ~ 5 years, and a pomelo tree will last longer than half. However, the quality of fruit is much worse.

3.6 Change planting time and density

Farmers in the Western Provinces historically grow citrus during the rain time from May to July. Recent studies showed that the density of citrus psyllid is usually very high during this period, but much lower in the period from October to December (Gottwald *et al*., 2010). Citrus planting should be conducted from October to December.

In the old way, the distance between trees was 2.5 m or less. Studies of the SOFRI-JIRCAS project showed that the planting of oranges at a distance of 3 ~ 4 m between trees combined with pruning and scattering resulted in better orchard management.

3.7 Chemical measures

When high population of psylla occurs during dry season, insecticides such as horticultural mineral oil, immidachoride-confidor, are suggested to be used 6 ~ 7 times during flushing time in Vietnam.

There is no drugs to eliminate HLB bacteria effectively in the tree, except in some cases the use of antibiotics for the disease control. The effects of penicillin and tetracycline in the control of HLB on the green grapefruit were determined. The results showed that the antibiotics did not leave residues in leaf at 8 months after treatment. The antibiotics had therapeutic effected to some extent on the disease, but the symptoms of the diseased plant disappeared temporarily, and about one year later they appeared again and even became more serious (Ke *et al*., 1988; Chen *et al*., 2014).

Submerging streptomyces into the soil or seedlings might be good to control fungal pathogens causing root rot, and create symbiotic microbes increasing the resistance to HLB. Most of the chemical insecticides are used to eradicate psyllid and it provides indirect protection against HLB infection. There is a need of further research for field application of pesticide irrigation to the roots of the tree.

3.8 Resistant varieties

In Vietnam there are some varieties of Rutacease family that are tolerance or resistant to HLB and ACP. Calamondin/kuk has good resistance to the disease, and Long Co Co Pumelo with a lot of hairs on the surface can hinder ACP to land and feed on this plant. This is a valuable source of genes for the breeding of resistance/tolerance in the future (Fig. 3).

Fig. 3　A lot of hairs on the skin of Long Co Co Pumelo

3.9 The causes of failure to prevent HLB in Southern Vietnam

The supply of disease-free seedlings to farmers is too limited compared to the high demand of production. The system of producing and supplying disease-free seedlings has not been organized and operated effectively due to various factors such as high capital infrastructure, high cost of seedlings, the farmers choice diseased seedlings with low price from private nurseries due to the government does not have a uniform policy to manage this.

There are a lot of extension activities with not effective, and disease prevention knowledge of farmers is low, they are spontaneous, lost confidence. Farmers are not aware of the harmful effects of HLB, so the application of preventive measures is too poor, sometimes without any measures. With the trees becoming diseased and dying gradually, they still keep the diseased trees to get harvest from them. Farmers apply high density planting and fast growing method, which is costly and profitable for only 2 ~ 3 years. The system of windbreaks around the orchards is not available or ineffective, not enough to prevent the HLB spread by ACP.

4 Conclusion

HLB is still a destructive citrus disease in Vietnam and China, and there are similar control measures against it, including removing diseased trees, controlling ACP, supplying organic fertilizer, using pest free seedlings, etc. However, there some techniques in Vietnam might be used for reference to control HLB and ACP in China, such as controlling the black ant for better cultivation of the weaver ants and interplanting of citrus with guava as staggered pattern. The Rutacease varieties with tolerance or resistant to HLB and ACP in Vietnam might be introduce to China.

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